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ANATOMICAL VARIATION IN THE AMOUNT AND COMPOSITION OF HUMAN SKIN SURFACE LIPID*

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ABSTRACT

Quantitative thin-layer chromatography was employed for determining the amount and composition of skin surface lipid from the forehead, cheek, chest, back, side, arm, and leg of adult male subjects. These data served as a basis for determining the relative contribution of epidermal and sebaceous lipids to the extractable surface lipid. The results indicate that lipid produced by the epidermis is an insignificant fraction of the total extractable surface lipid on areas rich in sebaceous glands, but can affect surface lipid composition on the limbs and on the trunk away from the midline. The amount of epidermal lipid recovered from the skin surface averaged between 5 and 10 μg per sq cm, compared with average recoveries of 150 to 300 μg of sebum per sq cm from the forehead.

The lipid extractable from the human skin surface with organic solvents is a mixture of sebum and of lipid produced by the keratinizing epidermis. On those areas with a high density of sebaceous glands the proportion of sebum in the surface lipid is obviously higher than where sebaceous glands are sparse. However, the relative contributions of lipid from the two sources have never been determined, although many workers have attempted to use variations in this ratio to predict the compositions of sebum and epidermal lipid (1, 2, 3). Recently, Wilkinson attempted to accentuate variation in the ratio of sebum and epidermal lipid by collecting surface lipid at various times after defatting the skin of the forearm (4). However, his results were not conclusive regarding the composition of the epidermal lipid or the relative contributions of epidermal lipid and sebum.

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We have reinvestigated the question of anatomical variation in the composition of skin surface lipids in order to determine the extent of the sebaceous and epidermal contributions to the surface film.

MATERIALS AND METHODS

Skin surface lipid was collected from five adult male volunteers 20 to 40 years of age, on two separate occasions. One group of three subjects washed thoroughly with Ivory® soap and water three hours before each collection, while the remaining two washed approximately twelve hours before the collections. The extraction sites included forehead, cheek, chest (sternum), back (interscapular), side (lowest rib), volar forearm, and the calf of the leg. Five ml of hexane was introduced into a glass cylinder of 10 sq cm cross-sectional area held firmly to the skin and the solvent withdrawn with a pipette. To facilitate quantitation the hexane contained known concentrations of methyl nervonate (10 or 50 μg/ml) as an internal standard. The recovered lipid solutions were evaporated with a stream of nitrogen and the lipid residue redissolved in 0.2 ml of hexane.

For analysis and quantitation by thin-layer chromatography, suitable volumes of the lipid solutions (3 to 20 μl) were applied to 7 mm wide lanes ruled on standard 20 × 20 cm glass plates coated with a 0.25 mm layer of Silica Gel G (E. Merck & Co.), as previously described (5). The plates were developed successively in hexane (to 19 cm), benzene (to 19 cm), and finally a mixture of hexane:ether:acetic acid (70:30:1, to 10 cm). The resolved lipids were charred by spraying with 50% sulphuric acid and heating to 220° C on a hot plate. When cool, the chromatograms were quantitated by scanning with a photodensi-

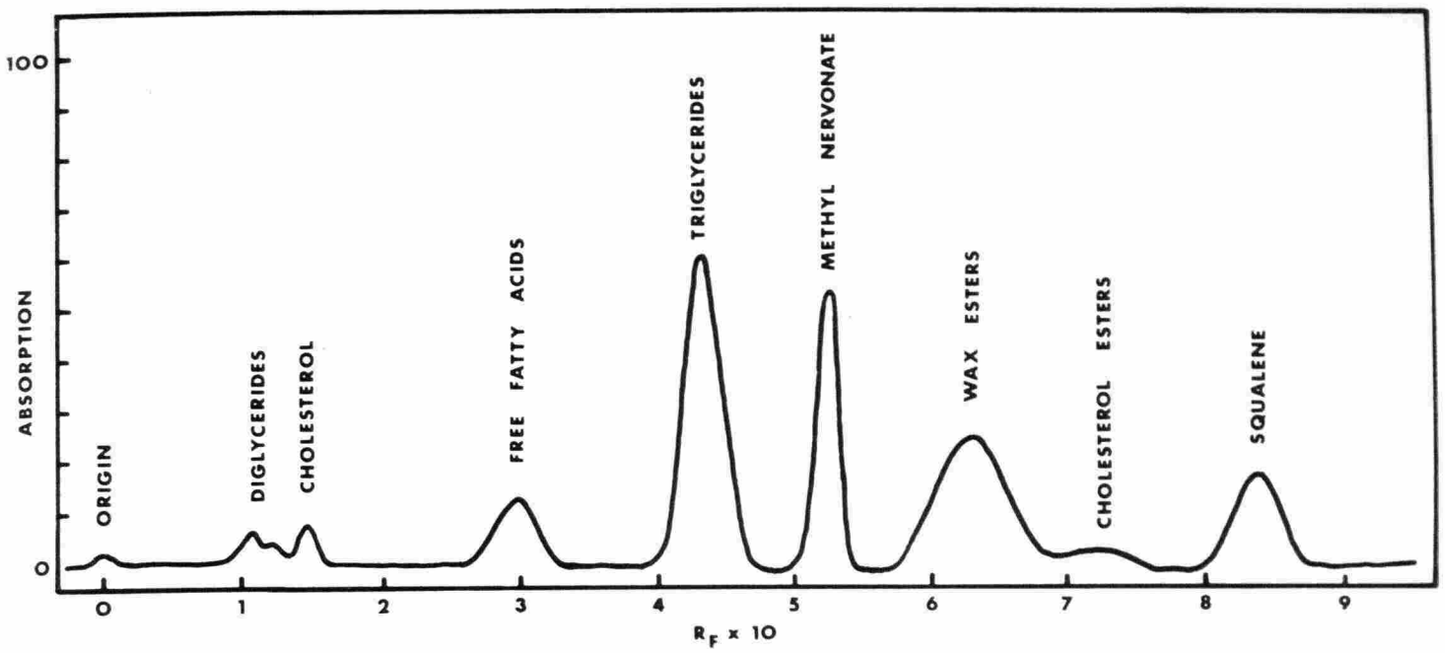

FIG. 1. Photodensitometer scan of a skin surface lipid sample, with methyl nervonate internal standard, resolved on a thin-layer chromatogram and charred.

tometer (Photovolt Model 52-C) attached to a strip-chart recorder (Varicord Model 42-B). Peak areas on the recorder chart, corresponding to the spots on the chromatograms, were determined by triangulation, and the data adjusted for the efficiency of charring of each class of compound (5). The relative amount of each lipid class in each chromatogram was calculated as a proportion of the total of the adjusted peak areas. In addition, the absolute amounts of lipid were determined by relation to the peak area for the methyl nervonate internal standard. Paraffin hydrocarbons, when present, were excluded from the calculations of composition and do not influence the results.

For graphical presentation of the data, slope and intercepts for straight lines were obtained by computer analysis by the method of least squares.

RESULTS

Figure 1 illustrates a typical photodensitometer record of the resolved skin surface lipids, together with the methyl nervonate internal standard. Each of the lipid classes was sufficiently well-resolved for adequate quantitation. The average amounts and composition of surface lipid recovered from each site for each of the two groups of subjects are shown in Table I.

For interpretation of the results of the analyses, the weight of each lipid sample was plotted against the weights of each of the major constituents in each sample. Separate graphs were prepared for each of the anatomical sites studied (Fig. 2). In carrying out this procedure the weights of triglycerides, diglycerides and free fatty acids in each sample were combined in a value termed "triglyceride fatty acids." This was done in order to eliminate variation due to differing degrees of hydrolysis of the triglycerides by bacterial or epidermal lipases. In each case, each series of points fell close to a straight line, the intercepts of which with the abscissae, computed by the method of least squares, are shown in Table II. With one exception (the forehead) the intercepts for triglyceride fatty acids were negative, while those for both wax esters and squalene were positive. The average values of the intercepts for wax esters and squalene fell in the range of 5 to 10 μg per sq cm. Since these compounds are generally considered to be produced by the sebaceous glands and not by epidermis, this result may be interpreted as meaning that no sebum would be present if the level of surface lipid was as low as 5 to 10 μg per sq cm. This, then, must represent the quantity of lipid contributed by the epidermis.

The negative value of the intercepts for triglyceride fatty acids implies that epidermal lipid contains a higher proportion of triglycerides than does sebum.

The exception to these results in the case of the forehead is believed to be a reflection of variability of the lipid composition rather than any inherent difference at this site. The effect of such variation can be reduced by averaging. Thus, the results of all of the seventy analyses may be combined by plotting the average

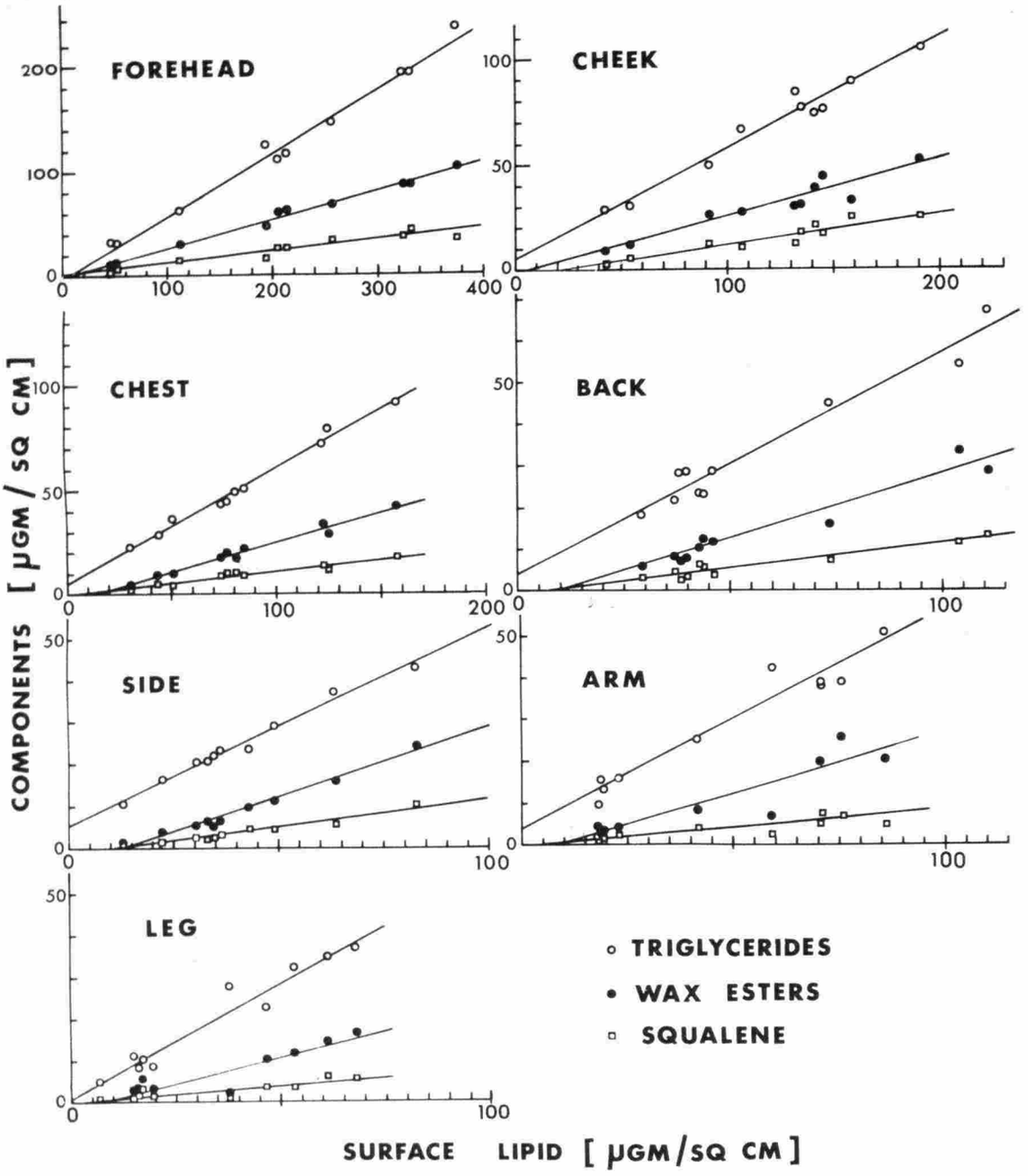

FIG. 2. Quantities of the major components of the skin surface lipids plotted against total amount of lipid in each individual sample from the anatomical sites shown.

weight of the constituents against the average quantity of surface lipid for each of the anatomical sites, as shown in Figure 3A.

To express more directly the relationship between the quantity and the composition of surface lipid, irrespective of anatomical origin, all of the results may be plotted individually on the one graph. This produced too many coincident points for presentation, but the abscissal intercepts obtained by computation on this basis are shown in Table II. To eliminate overlapping, the results may be averaged for ranges of weight of recovered surface lipid, as shown in Figure 3B. The abscissal intercepts computed for this treatment are also given in Table II.

The results may also be presented in the more usual form of a graph of percentage composition plotted against weight of recovered lipid. This method of expression provides additional insight, as shown in Figure 4, where the results are averaged for each anatomical site, for the three-hour (4A) and the twelve-hour (4B) accumulation periods. It is apparent that with increasing quantities of surface lipid (reflecting greater contributions of sebum) wax esters and squalene increase, while the proportions of cholesterol and cholesterol esters decrease. The proportion of each of the constituents becomes nearly constant when the amount of total lipid reaches 50 to 100 µg per sq cm, indicating that the influence of

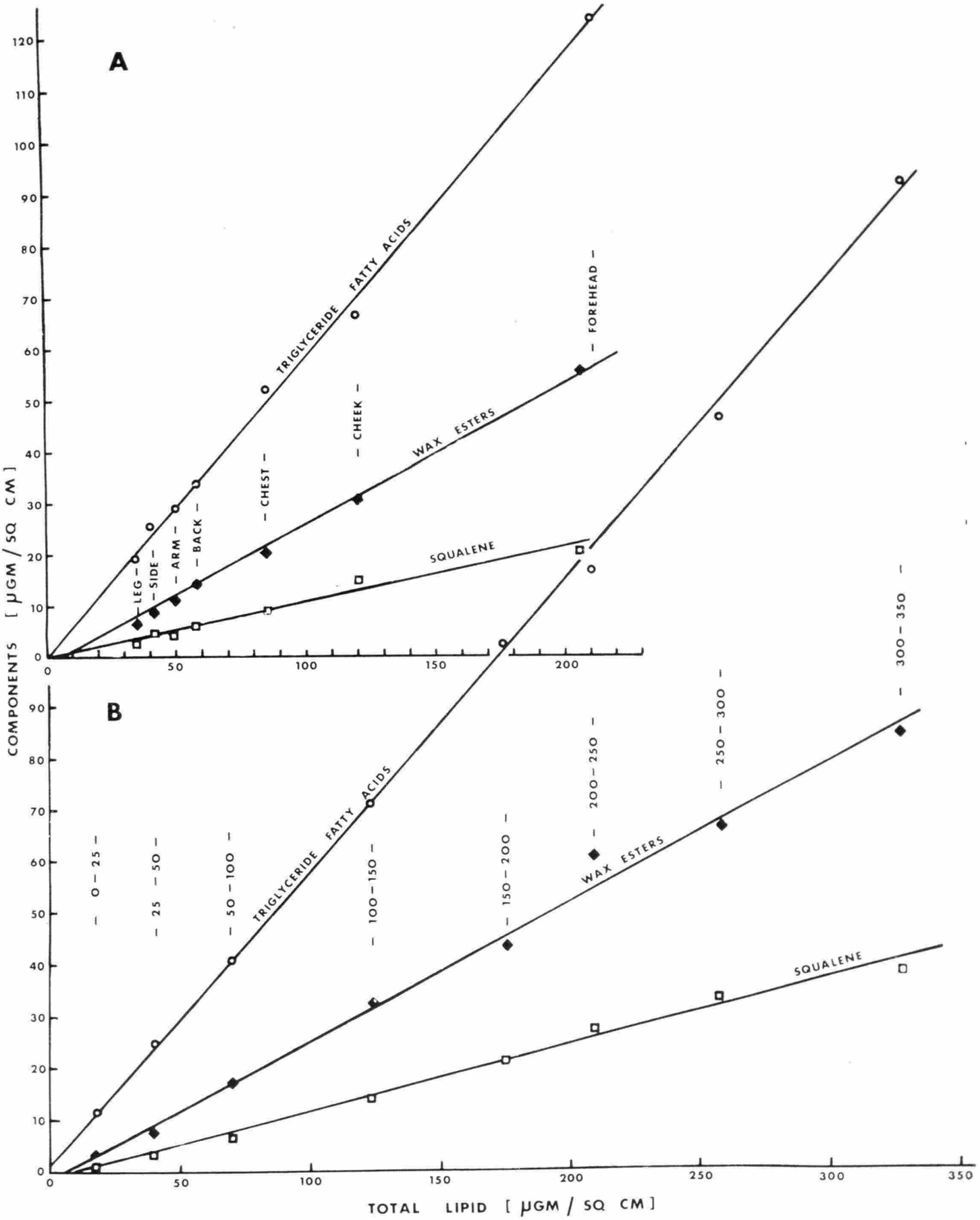

FIG. 3. Average quantities of lipid components from different anatomical sites plotted against the average total lipid recovered from the respective sites. (Combined results for five subjects, each extracted on two occasions.) B. Average quantities of lipid components in successive ranges of total lipid extracted, plotted against the average quantities of total lipid in each of the ranges shown. (Combined results for five subjects, each extracted on two occasions.)

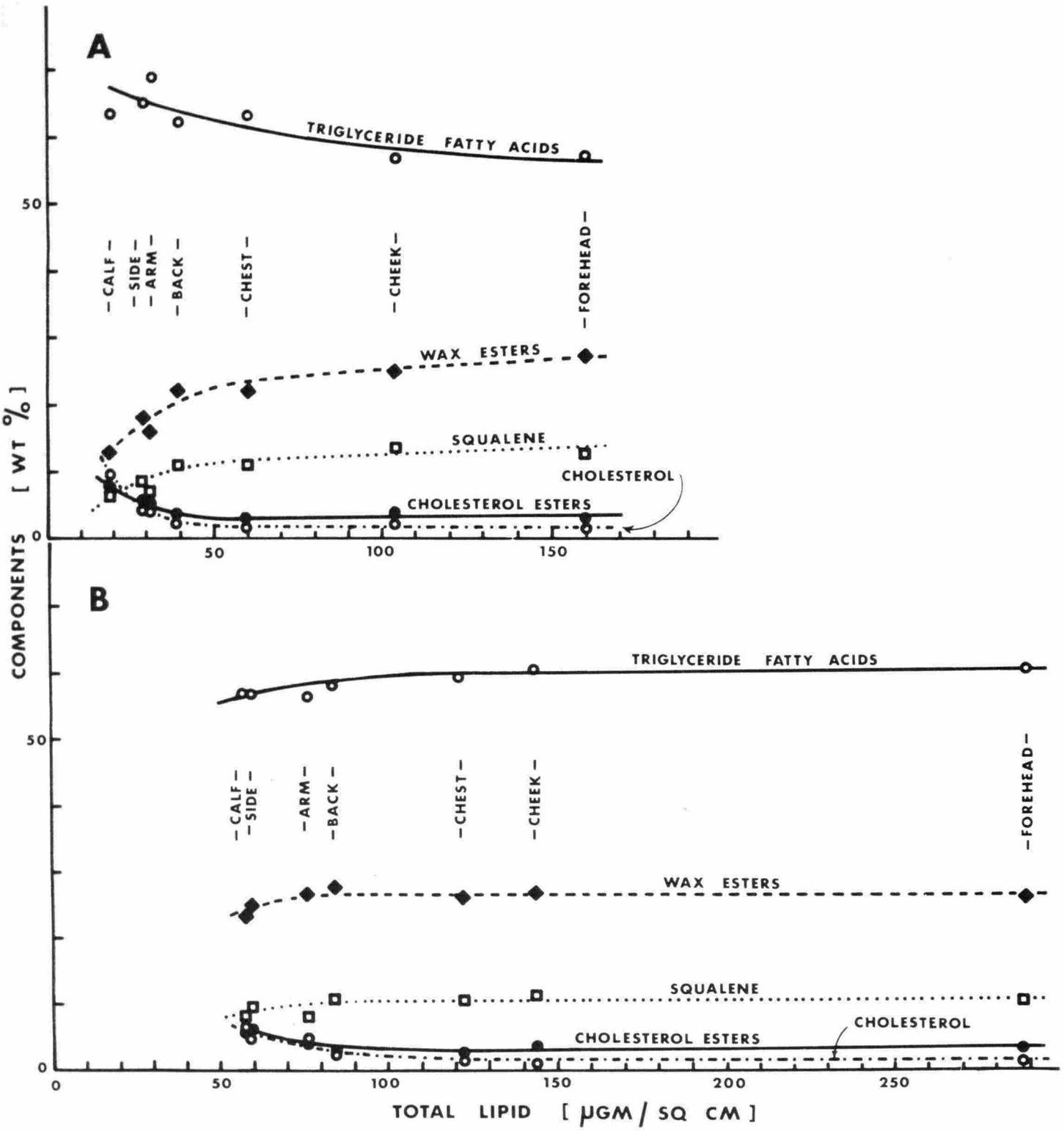

FIG. 4. Average composition of skin surface lipids from different anatomical sites plotted against the average total lipid recovered from the respective sites. A. Results for three subjects washed three hours prior to extraction of the surface lipids on each of two occasions. B. Results for two subjects washed twelve hours before extraction of surface lipids, each on two occasions.

epidermal lipid becomes insignificant above this range. The fact that the proportions of cholesterol and cholesterol esters do not continue to fall at higher levels of surface lipid accumulation is evidence that these are also constituents of sebum, but at lower concentrations than in epidermal lipid. This interpretation is reflected in the increase in amounts of cholesterol and cholesterol esters with increasing lipid accumulations shown in Figure 5 and the negative abscissal intercepts computed for the straight lines in that graph.

The composition of the lipid at high levels of accumulation may therefore be accepted as the approximate composition of pure sebum. The average composition of the lipid samples recovered in amounts greater than 100 μg per sq cm is given in Table III.

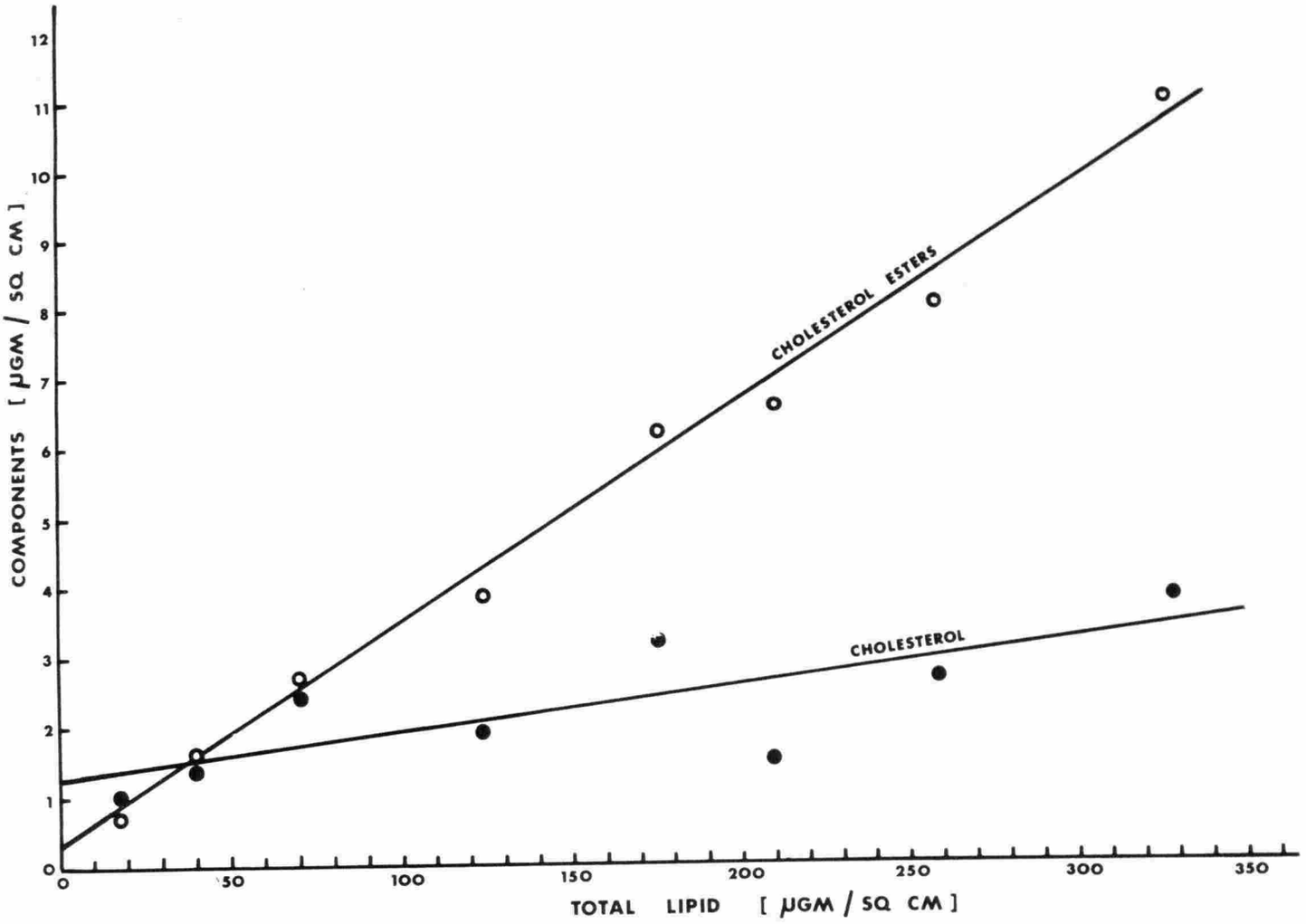

FIG. 5. Average quantities of cholesterol and cholesterol esters in different ranges of total lipid extracted, plotted against the average quantities of total lipid in the same ranges as in Figure 4B. (Combined results for five subjects, each extracted on two occasions.)

DISCUSSION

While the composition of human skin surface lipid has been studied by many workers, interpretations have been inhibited by lack of knowledge of the relative contributions of sebum and epidermal lipid. The present study indicates that the quantity of extractable epidermal lipid is between 5 and 10 μg per sq cm, compared with 150 to 300 μg of sebum per sq cm on the forehead. The epidermal lipid, therefore, contributes about 3 to 6% of the surface lipid on the forehead. The influence of epidermal lipid on surface lipid composition is diminished by virtue of the fact that its major constituent, triglyceride, is present in approximately the same proportion in sebum. As a result, the contribution of epidermal lipid does not produce a significant effect on composition unless the quantity of surface lipid is less than about 100 μg per sq cm. Thus, when sufficient time is allowed for its accumulation the composition of the surface lipid is similar for the forehead, face, chest and back. Significant deviations may occur on the limbs and on areas of the trunk away from the midline at short periods of accumulation. In unpublished studies, we have induced reductions of up to 70% in sebum production without detectable change in the composition of surface lipid of the forehead. This again demonstrates the slight epidermal contribution.

It seems probable that washing with soap and water failed to extract an appreciable proportion of the lipid in the stratum corneum, because of the known effectiveness of this tissue as a barrier to water. Furthermore, the use of a water-immiscible solvent for the extractions probably prevents penetration to the moist levels of the epidermis and limits extraction to the desiccated surface layers. Thus, the quantities of epidermal lipid recovered are unlikely to be related to the total amount of

TABLE I

Anatomical variation in amount and composition of human skin surface lipid

Site	Total lipid (μg/sq cm)	Composition (weight percent) CH	CE	TG	DG	FA	WE	SQ	TG & DG & FA
A. *Three-hour accumulation*									
Forehead	160	1.2	2.9	30.3	2.3	24.1	27.0	12.3	56.7
Cheek	104	2.0	3.9	20.9	2.9	22.4	24.3	13.5	56.2
Chest	59	1.6	2.9	32.7	2.3	28.1	21.7	10.7	63.1
Back	38	2.1	3.0	34.5	2.7	24.9	21.9	10.7	62.1
Arm	30	4.1	4.4	30.7	1.5	36.4	15.8	6.9	68.6
Side	29	3.9	5.3	39.2	1.9	23.4	17.7	8.5	64.5
Leg	19	9.4	7.5	24.2	1.8	37.8	12.9	6.2	63.8
B. *Twelve-hour accumulation*									
Forehead	288	1.1	2.7	29.6	3.5	27.2	25.9	10.1	60.3
Cheek	144	1.1	3.4	39.4	2.7	15.4	26.9	11.2	57.5
Chest	122	1.3	2.6	29.7	5.4	24.9	25.7	10.3	60.0
Back	84	2.2	2.0	35.9	4.5	17.4	27.4	10.6	57.8
Arm	76	4.8	4.3	34.3	2.4	18.4	27.7	8.1	55.1
Side	57	4.3	4.5	47.1	1.9	7.6	24.9	9.6	56.6
Leg	57	6.3	6.0	44.6	1.5	10.2	23.1	8.1	56.3

TABLE II

Intercepts on the abscissae, calculated by the method of least squares

Site	Triglyceride fatty acids	Wax esters	Squalene
	(micrograms per sq cm)		
(a) *for Fig. 2*			
Forehead	2.9	3.0	−11.5
Cheek	−11.8	6.6	23.0
Chest	−8.3	14.4	5.6
Back	−7.1	11.5	4.8
Side	−11.8	13.4	11.6
Arm	−6.8	10.0	0.1
Leg	−2.0	7.4	2.8
Average	−6.4	9.9	5.2
(b) *for all sites combined*			
(i) individual analyses	−4.2	4.9	16.5
(ii) weight ranges (Fig. 3B)	−2.7	5.5	9.2

TABLE III

Approximate composition of sebum (average composition of surface lipid samples recovered in amounts greater than 100 μg/sq cm)

Constituents	wt. %
Triglycerides	57.5
Wax esters	26.0
Squalene	12.0
Cholesterol esters	3.0
Cholesterol	1.5

lipid present in the full thickness of the epidermis.

It is of interest to note, however, that the average quantity of lipid recovered from the forehead after twelve hours (288/μg sq cm) agrees with the value of 280 μg reported by Kirk (6) for the same accumulation period for males in a similar age range. Kirk reported a recovery of 224 μg/sq cm from the forehead after a four-hour period, compared with 160 μg for three hours in the present study.

With the low levels of lipid accumulation en-

countered, the question of contamination by exogenous lipids must be considered. In this study such contamination was minimized by the thorough washing of the extracted areas immediately prior to the accumulation period. Ivory soap was employed for this purpose since it does not contain added lipids, and consists almost exclusively of the sodium salts of fatty acids. Triglycerides, cholesterol, and cholesterol esters, which we find to be the principal constituents of epidermal lipid, are not present in the soap in significant amounts. The presence of paraffin hydrocarbons, which have often been reported as contaminants of human skin, would not influence the present results since these lipids are resolved from the lipid classes present in the skin and have been excluded from our calculations.

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